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A Case of Sleeping Sickness Studied by Precise Enumerative Methods: Regular Periodical Increase of the Parasites Disclosed.

BY MAJOR RONALD ROSS, F.R.S., AND DAVID THOMSON, M.B., CH.B., D.P.H.

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A Case of Sleeping Sickness Studied by Precise Enumerative Methods: Regular Periodical Increase of the Parasites Disclosed.

By Major RONALD ROSS, F.R.S., and DAVID THOMSON, M.B., Ch.B., D.P.H.

(Received May 2,—Read June 16, 1910.)

Prefatory Note by R. Ross.—For a long time it has appeared to me that much light might be thrown on infectious diseases, immunity and treatment, by more exact enumeration of the infecting organisms, and that we might even be able ultimately to apply mathematical reasoning to the study of these subjects. In 1903* I elaborated a method of blood examination, called the *thick-film process*, which enables us to detect small organisms in the blood about twenty times more easily than in ordinary preparations; but for the lack of the necessary assistance I was long unable to apply the method to the laborious enumeration of such organisms. Recently, however, the Advisory Committee for the Tropical Diseases' Research Fund has placed considerable funds at the disposal of the Liverpool School of Tropical Medicine for the study of cases in the tropical ward of the Royal Southern

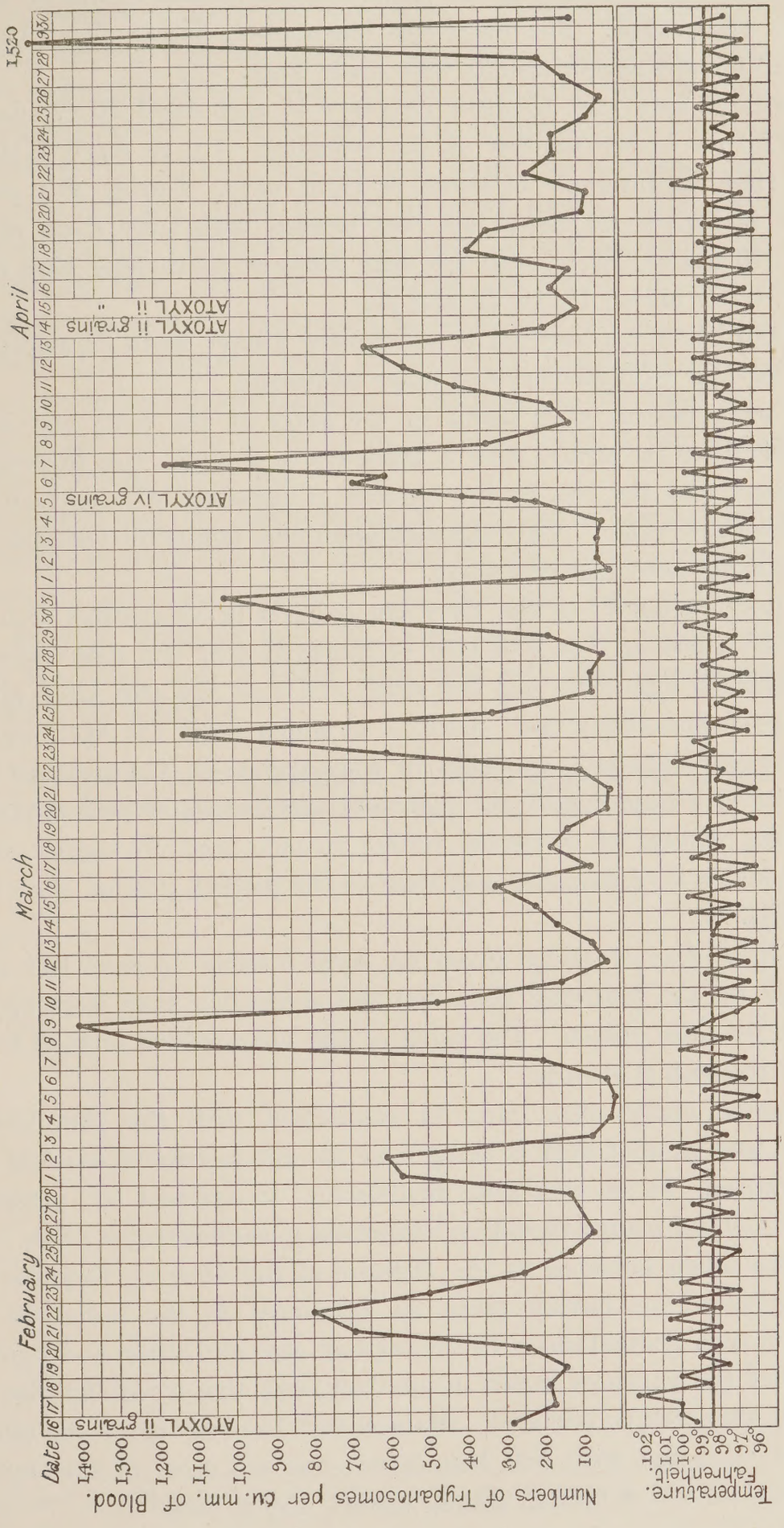
* 'Lancet,' January 10, 1903, and Thompson Yates Reports, vol. 5, Part I, 1903.

Hospital, Liverpool—with the result that the investigations referred to were commenced by Dr. David Thomson and myself from the beginning of this year. As I expected, methodical counting of the parasites has at once verified or disclosed several facts of importance in connection with malaria and trypanosomiasis. We now limit ourselves to a brief description of the remarkable periodical increase of *Trypanosoma gambiense* revealed by careful daily counting in a case in my clinic (the case, which is still under treatment, will be fully reported elsewhere).

W. A., male, aged 26 years, a strong young man born in Northumberland, was infected in N.E. Rhodesia in September, 1909, the trypanosomes being found in his blood in Africa on November 17. On coming to Liverpool for treatment he was admitted into the Southern Hospital on December 4. From then until February 16 (73 days) the number of trypanosomes in his blood was estimated only by the rough methods in common use; that is, by the proportion of trypanosomes to red cells or leucocytes, or to "fields" of the microscope examined. These methods are obviously open to such great error that they can scarcely be depended upon to indicate any but very large differences in the numbers of objects counted. During the 73 days 46 counts were made; but on several occasions none was attempted for three or four days in succession—so that, even if the methods of counting employed had been more accurate, sudden fluctuations might easily have been missed. Hence, as was to be expected, the graph during this period is very irregular and almost worthless. On admission on December 4 the patient was reported to contain about 6000 trypanosomes per cubic millimetre of blood, and large numbers, amounting to about 3000 per cubic millimetre, were found on December 17 and 28 and on January 16. All this time the patient was given the usual treatment with atoxyl and mercury, and received altogether 10 doses of two to four grains of the former. Nevertheless the parasites never fell below about 200 per cubic millimetre in number, as roughly estimated.

It was then found, however, that atoxyl was injuring the patient's sight (as sometimes happens), and other treatment was substituted. At the same time we elaborated a much more correct method of counting all the parasites in measured quantities (one quarter to 1 cubic millimetre) of blood taken in thick film; and from February 16 onward the patient's trypanosomes were estimated daily by this method by one of us (D. T.). The attached chart gives the remarkable graph obtained up to the present (April 30).

The numbers of trypanosomes found were scrupulously recorded, and the


Numbers of Trypanosomes per cu. mm. of Blood.
Temperature. Fahrenheit.
Date
February
March
April
1520
ATOXYL ii grains
ATOXYL iv grains
ATOXYL ii grains
ATOXYL ii "

smoothness and regularity of the graph suggest that there was no very great error of observation. The blood was taken every day at about 10 A.M., but on April 5 and 6 several counts were made daily.

It will be seen that between February 16 and April 30 (73 days) there were 11 rises in the number of the parasites. Up to April 7 there were seven rises, at intervals of seven or eight days. During this period the patient was given no atoxyl, but was treated with large doses of quinine (30 to 40 grains) daily, with frequent doses of methylene blue, and with trypan red on March 17, 18, 19, and 20.

On April 5 it was decided to administer atoxyl again as shown on the chart, together with mercury and other treatment.

The temperatures were taken by the sister of the ward, and it will be seen that there has always been a tendency to a slight rise in temperature concurrent with the rise in the number of parasites—the two curves thus confirming each other. Only the maximum and minimum temperatures are entered in the accompanying chart.

The great regularity of the rises can scarcely be compatible with a mere chance distribution. It will also be observed that the rises were of two kinds, namely, high rises and low rises; and that the two kinds alternated with regular periodicity until April 18, at which point the cycle appears to have become distorted—probably in consequence of the treatment. The regularity of alternation of the high and low rises is so well marked as to recall the picture of a double tertian malaria, and to suggest that two independent sets of parasites may exist in the patient, just as often happens in malaria.

The large dose of atoxyl given on April 5 seems to have had no effect whatever on the following rise; but the succeeding rises were apparently modified for some reason. The value of the enumerative method for therapeutical research is obvious.

Of course, many other facts in connection with the case have been recorded, and parallel work is being done on sub-inoculated animals and on the parasites. It is, therefore, inadvisable to attempt at present any discussion of the many interesting theoretical questions which arise.

We are much indebted to the Director of the Sleeping Sickness Bureau (Dr. Bagshawe) for having given us references to the literature on the subject of such fluctuations. In the original case of Dutton and Ford it was noted that the parasites varied in numbers, and that a parallel rise in the patient's temperature occurred. Manson and Daniels* chart the number of parasites compared with 500 leucocytes; but the error of this method is

* 'British Medical Journal,' May 30, 1903.

very large and their graph is quite irregular. They abandoned counts in measured quantities of blood as "unreliable." Thomas and Breinl* showed that in three cases of Sleeping Sickness the numbers of trypanosomes found in "fresh cover-slip preparations" varied irregularly from time to time. Koch, Beck, and Kleine (1909) remark on the irregularity of the appearance of *T. gambiense* in African natives, and state the parasites are present for two to five days and absent for two to three weeks. Salvin-Moore and Breinl† show a graph with two undulations and a final premortal rise in two heavily infected rats, and give a detailed description of corresponding changes in the parasites. Apparently, hitherto, only irregular variations in the numbers of the parasites seem to have been recognised; probably the large error due to inadequate methods of counting has disguised the regular periodicity of the variation shown by more exact counts in the 11 successive undulations observed in our case.

We should add that our methods enable us to detect parasites when they are in numbers so small that their detection by the ordinary methods would be exceedingly laborious. Hence if our case had been studied by the ordinary methods, probably only the crests of the rises would have been visible in the chart, and it would have been said that the parasites had disappeared in the intervals.

* 'Memoirs of the Liverpool School of Tropical Medicine,' vol. 16, 1905.
† 'Annals of Tropical Medicine, Liverpool,' vol. 1, No. 3, 1907.

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